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FACTORS IN HEMOLYSIS.*†

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DURING an investigation in hemolysis, extending over about three years, a number of unsuspected phenomena have been encountered. The discovery of these phenomena has modified the original comparatively simple concept of hemolytic serum, so that in place of the two components at first regarded as the only active substances in such serum, at least a dozen components are now recognized.[1] Some of these components have been found under certain conditions to be beyond experimental control, and unrecognized, to introduce serious error in hemolytic investigation.

The experimental work, of which this paper is in a way a summary, was done with goat serum immunized against sheep corpuscles. The substances herein enumerated, therefore, must be regarded as found only in such serum. These substances are:

1. AMBOCEPTOR. A specific, thermostable substance, or group of substances, formed in goat serum in response to repeated injections of washed sheep corpuscles. This substance is not destroyed by heating to 60° C. for several hours, or by standing at ordinary temperatures for months. Amboceptor is a necessary factor for the hemolysis of sheep corpuscles by goat serum; hemolysis not taking place in its absence, regardless of the number or the amount of the other serum components present.

The action of amboceptor is not understood. The current belief that it is absorbed by corpuscles rests on doubtful experimental grounds, as the quantitative analysis of an amboceptor containing goat serum that has once been in contact with sheep corpuscles is at present out of the question.[2] One can only say that contact with amboceptor in some way renders sheep corpuscles susceptible to

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[1] For techni., see *Jour. Infect. Dis.*, 1905, 2, p. 461.

[2] *Jour. Infect. Dis.*, 1907 4, p. 219.

hemolysis by certain other components of goat serum, which to unexposed corpuscles are non-hemolytic. No trace of amboceptor is demonstrable in normal goat serum.

2. COMPLEMENT. A non-specific, thermolabile substance, or group of substances, found in normal goat serum. Whether or not the amount or nature of the complement is changed, as a result of repeated injections of sheep corpuscles, is not known. Goat complement is apparently completely destroyed by heating to 50° C. for 60 minutes, to 55° C. for 15 minutes, or to 60° C. for 5 minutes.[1]

Complement is a necessary factor for the hemolysis of sheep corpuscles by goat serum; hemolysis not taking place in its absence, regardless of the number and amount of the other serum components present. In itself complement is non-hemolytic, requiring for its action the presence of a certain amount of amboceptor. Sheep corpuscles, however, that have been exposed to goat amboceptor are readily hemolized by complement alone. The nature and the method of action of complement are not understood.

3. THIRD COMPONENT. A collective name applied to the other constituents of goat serum. The nature of these constituents is impossible of experimental study in unheated sera, due to the presence of the hemolytically more active complement. A study of normal goat serum in which the complement has been destroyed by heat, however, shows that the third component then apparently consists of at least three hemolytically active substances, or groups of substances. These are: (1) an antilysin, or hemolysis-inhibiting substance, and (2) two auxilysins, or hemolysis-increasing substances.[2]

The nature of these substances is not understood. Whether they are independent substances or not, and whether or not they are derivatives of complement, that is, "complementoids," have not been determined. The amounts of these substances apparently differ considerably in sera from different goats, and in the serum of the same goat at different times and under different experimental conditions. Their chemical composition, however, is apparently the same in different sera.

The third component in itself is non-hemolytic, and is incapable of producing hemolysis in the presence of pure amboceptor or pure complement. It is believed that many current errors in hemolytic theory are due to failure to take into account the presence of, and variations in, this component.

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[1] *Trans. Chi. Path. Soc.*, 1906, 6, pp. 425–27.

[2] *Jour. Infect. Dis.*, 1906, 3, pp. 225, 226, 647.

4. CORPUSCLE EXTRACT. Washed sheep corpuscles probably give off into the surrounding medium at least three substances capable of modifying hemolysis. These are: (1) traces of sheep complement, whose action is apparently identical with that of goat complement, (2) traces of sheep third component, whose action is believed to be similar to that of goat third component, and (3) certain substances believed to be products of corpuscle autolysis.[1]

The principal active component in these autolytic products is a powerful, antilytic, thermostable substance, whose nature is not understood. It is the presence of this substance that renders the quantitative analysis of amboceptor-containing goat serum impossible, after the serum has been exposed to sheep corpuscles. Whether or not similar substances are given off into goat serum from its own corpuscles, has not been determined.

5. HEMAGGLUTININ. A specific, thermostable substance, or group of substances, formed in goat serum in response to repeated injections of sheep corpuscles. The amount of this substance varies greatly in different sera. One serum may produce complete hemagglutination in a few minutes, while another serum of apparently equal hemolytic power may be practically without agglutinating action.

The nature of this hemagglutinin is not understood. Whether or not it bears any chemical relation to amboceptor, is unknown. Its effect on hemolysis is believed to be to decrease lysis, by clumping corpuscles and thus removing them from the action of amboceptor and complement. No trace of hemagglutinin is found in normal goat serum.

6. ACCESSORY HEMAGGLUTININS. At least two substances, or groups of substances, have been encountered that modify the rapidity or the completeness of the agglutinating process. These are: (1) an auxagglutinin,[2] or agglutination-increasing substance in the third component, and (2) an antiagglutinin, or agglutination-decreasing substance in corpuscle extract. The nature of these substances is not

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[1] *Jour. Infect. Dis.*, 1907, 4, p. 219.

[2] *Ibid.*, 1906, 3, p. 227.

understood, and their relation to the other active substances in third component and corpuscle extract is not known.

7. CORPUSCLE SUSCEPTIBILITY. Changes in the susceptibility of washed sheep corpuscles, both to hemolysis and to hemagglutination, have been observed. The nature of these changes is not understood, and their importance in hemolytic work is undetermined. It is possible, however, that they introduce error in certain hemolytic investigations.

8. INORGANIC SALTS, SPECIFIC GRAVITY, REACTION OF MEDIUM. Certain inorganic constituents of serum, especially the calcium salts, are known to be powerfully autilytic.[1] The influence of specific gravity on hemolysis is not accurately known, but it is possible that errors may arise from variations in it. The reaction of the medium, that is, its degree of acidity or alkalinity, is known to influence greatly its hemolytic action. The importance of these three factors, however, in hemolytic investigation, has not been adequately determined.

9. HEMOPSONIN. It is suggested by Dr. Hektoen that the corpuscles in a hemolytic tube might possibly become opsonized by the hemopsonins[2] of the immune goat serum, and that some of them might be taken up by the leucocytes of the blood suspension and protected from hemolysis. Numerous smears made from routine hemolytic tubes, however, show no sign of such phagocytosis.

In the routine technique, the corpuscles are 24 hours old. The above examination, therefore, does not exclude the possibility of phagocytosis playing a rôle in hemolytic experiments under a technic involving the use of fresher corpuscles. Such a rôle, however, must be practically negligible, as it is inconceivable that the leucocytes could take up more than a maximum of ¼ per cent of the red corpuscles present, which would introduce a maximum error in the hemoglobin liberation less than the minimum difference in tint that can be detected by ordinary colorometric methods.

10. PRECIPITINS. It is further conceivable that specific precipitins may have an influence in certain hemolytic experiments. No trace of such precipitins, however, has been found in goat serum immunized against washed sheep corpuscles or against normal sheep serum.

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[1] *Jour. Infec. Dis.*, 1904, 1, p. 379.

[2] *Ibid.*, 1906, 3, pp. 721

SUMMARY

The substances above enumerated may be tabulated, as follows:

- I. NECESSARY FACTORS
  - 1. Amboceptor
  - 2. Complement
- II. ACCESSORY FACTORS
  - 3. Third Component
    - (1) Antilysin
    - (2) Auxilysin I
    - (3) Auxilysin II
  - 4. Corpuscle Extract
    - (1) Complement
    - (2) Third Component
    - (3) Autolytic Products
  - 5. Hemagglutinin
  - 6. Accessory Hemagglutinins
    - (1) Serum Auxagglutinin
    - (2) Corpuscle Antiaggultinin
  - 7. Corpuscle Susceptibility
  - 8. Inorganic Salts
  - 9. Specific Gravity
  - 10. Reaction of Medium

In enumerating these components, no claim is made that they are all independent substances. Nothing is known of their chemical composition, nor of the relations existing between them. Their enumeration, however, is believed to be of value, as it indicates the kind of conplexities that may be expected in other fields of serum pathology.